How to Get Good Results

What is Auto-Defrost & Drain?

Auto-Defrost and Drain is an option for the standard condensers on Genevac HT-4X, HT-8/12 series 2 and HT-24 Workstation. Auto-Defrost and Drain enables the system to automatically drain the condenser of volatile solvent(s) between stages in a method, and at the end of the method to fully defrost and drain the system with no user intervention. When performing an intermediate drain (i.e. mid method) the system also does a short defrost just in case residual solvent from the previous use has frozen in the outlet pipe of the condenser. The intermediate drain can only be used to remove volatile solvents which do not freeze at temperatures above –50°C.

Benefits of Auto-Defrost & Drain

The principal benefit of the Auto-Defrost and Drain function is to eliminate volatile solvent(s) from the condenser. Volatile solvents boil off first from mixtures and are collected in the condenser. To remove the higher boiling point solvents, low pressures must be achieved later in the process. However, dropping the pressure causes the previously condensed volatile solvent to re-boil in the condenser, which generates a very large volume of vapour which must exit through the pump. Until all this vapour has been pumped away (which can take several hours) the system cannot achieve a lower pressure and so the higher boiling point solvents cannot be boiled. The volatile solvents are therefore said to 'spoil' the vacuum level. Vacuum spoiling affects final dryness of samples, or in the very worst cases, the ability to evaporate the higher boiling point solvent at all. To overcome these problems the user always had to, until now, be present to drain the condenser after the volatile solvents had been captured in the condenser. This draining operation can now be automated. An additional benefit of Auto-Defrost and Drain is that volatile solvents are collected and can be safely disposed of, reducing VOC emissions.

If your evaporator is equipped with the Auto-Defrost and Drain facility you will see the "Lyo Speed Enabled" sign on your systems and the Auto-Defrost and Drain option will appear in the run menu when programming methods.

Flexibility

A Genevac evaporator enhanced with Auto-defrost and Drain functionality can not only be used as part of the LyoSpeed™ process (see overleaf), in addition it will also deliver improved results when working with any mixture of solvents with differing boiling points.

When evaporating HPLC fractions, auto-defrost and drain will help achieve excellent final dryness. Mixtures of DCM and DMSO or DMF, are almost impossible to evaporate without draining the cold trap once the DCM is removed, and before tackling the higher boiling point solvent. This is now possible with a system which is Auto-defrost and Drain enabled.

Genevac technology is protected by patents and patent applications in the UK and worldwide.

How it Works

The auto-defrost and drain enhanced condenser has the following features:

- Enhanced condenser draining with minimum hold-up design
- Automation of the drain valves
- Specific defrost of condenser outlet pipe
- Mid-method short defrost and drain to remove volatiles
- Automated full defrost and drain at end of method

Auto-Defrost and Drain will appear as an option in the programming page when entering a method. Select the method to enable it – for details see the programming guide in the user manual. The evaporator will perform in one of two ways depending on whether the method is part of a linked series of methods:

- If the method is stand alone and not linked to any other method, then when the evaporation method has ended the system will do a full defrost and then drain condenser automatically.
- If the method is linked, and has other methods after it, then at the end of the method the system will perform a short defrost sufficient only to thaw any frozen solvent trapped in the outlet pipe, then drain the condenser. When this is complete, it will automatically continue with the next linked method
- If the method has other methods linked to it, but is the last in the chain, then at the end of the method the system will do a full defrost, and drain the condenser automatically.

When to use Auto-Defrost and Drain

Auto-Defrost and Drain has two main functions:

1. to automatically drain the condenser mid-process to eliminate volatile solvents, enabling the system to reach full vacuum in the later stages of evaporation. This allows the system to achieve better final drying of the sample, and to get better evaporation of mixtures of solvents with widely differing boiling points.
2. to automatically defrost and drain the condenser at the end of the method, saving the user time. This means that for an overnight method which finishes before the user comes to the Lab in the morning, the system has defrosted, drained and is ready to use straight away. This has a benefit for any solvent.

The second of these requires no further explanation, however, there are a number of applications for the first application – the intermediate defrost and drain. These include:

- Drying of HPLC fractions where high vacuum in the final stages of evaporation is beneficial
- Use of the Genevac fast lyophilisation method for HPLC fractions, where high vacuum in the latter stages of the process is essential
- Evaporation of DMF or DMSO mixed with a volatile solvent, e.g. dichloromethane (also known as DCM or methylene chloride) where the mixture of solvents necessitates high vacuum for the high boiling point solvent which is removed last, but where the volatile solvent removed first will sit in the condenser and spoil the level of vacuum which can be achieved in the latter stages of the run.

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When not to use Auto-Defrost and Drain

There are certain circumstances when Auto-Defrost and Drain should not be used. This is in order to protect your system and/or your samples from solvent damage. Do not use Auto-Defrost and Drain in the following circumstances:

- Evaporation of TFA - intermediate (short) defrost and drain is permitted. Full defrost and drain at the end of the method should not be done if there is residual TFA in the condenser. Perform a manual defrost for 10 minutes, manually terminate defrost, manually drain the system
- At the end of a lyophilisation method – water vapour may travel back from the cold trap to the chamber and cause the powders to collapse. Remove the samples first, then defrost and drain.

Drying HPLC Fractions

The best optimised evaporation method has the following stages:

1. **First Stage** - removal of the organic phase
    a. Use Variable Dri-Pure™ to prevent bumping:
    Ramp the pressure down from 175mbar to 40 mbar (acetonitrile) or 45mbar (methanol) in 20 minutes at high rotor speed (500g).
    Control the pressure at 40mbar for Acetonitrile and 45mbar for Methanol - these control pressures are chosen to keep the boiling point above 0^{o}C (i.e. don't freeze the water).
    b. Auto-Defrost and Drain at the end of the stage – this will be a short defrost (just in case any small amounts of water have frozen in the drain port) and will drain the organic solvent from the condenser, allowing high vacuum levels to be achieved later on in the run.
    c. Consult your local Genevac representative for advice on the evaporation time for this stage. However, a good rule of thumb is that for 50/50 Acetonitrile water the overall length of this stage should be approximately 1/3 as long as the length of stage 2 (which the system will determine automatically).

2. **Second Stage** - removal of the aqueous phase
    a. Control the pressure at 8mbar (which ensures the BP stays above 0^{o}C and the water cannot freeze)
    b. Keep the rotor speed high (promoting good convection & hence heat flow in the solution)
    c. End the method using "Heat Flow", rather than specifying a duration for the stage.

3. **Third Stage** - drying the stubborn samples.
    a. Run the system at Full Vacuum for 1 to 3 hours
    b. Trial and error will be required to optimise this stage. Different compounds will require more of less of this to reach final dryness, with highly polar molecules often taking longest.
    c. Auto-Defrost and Drain at the end of the stage - this will be a full defrost and drain, as there are no more stages to follow.

See appendix A for programming details.

Please contact your local Genevac representative for further details on run times, alternatively please e-mail Applications@Genevac.com

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Lyophilising HPLC Fractions

The best optimised evaporation method has the following stages:

1. Follow stage 1 of the 'Drying HPLC Fractions' method to remove the organic solvent
2. Cool the samples – concentrating some of the water without any heat input
   Use controlled pressure 8mbar to boil away some of the water without any heat input, this will cool the holders and help achieve good freezing in the next stage. It may be possible to concentrate more water at this stage – if you have very large or water heavy fractions please contact Genevac for advice
3. Freeze all the samples using high vacuum
   Run the system at Full vacuum with no heat for 1 hour
4. Lyophilise the samples to dryness, using heat if possible to speed the process up
   Run the system at full vacuum, trial and error will determine the length of time that Lyophilisation takes

See appendix B for programming details.

Please contact your local Genevac representative for further details on run times, alternatively please e-mail Applications@Genevac.com

Notes on Lyophilisation

- Setting up the fast lyophilisation method takes some trials to establish a fully functioning process, it may take several attempts. It is a very useful to have the Genevac Data Logging software running on an adjacent laptop or PC to collect the evaporation data which can then be sent to Genevac as an aid providing remote assistance.
- Always use both sample guard probes, placing one in the sample holder to control the temperature, and one in a central sample to provide feedback on the process. This is essential during method development, this will tell you if your sample has frozen or not, and when lyophilisation has ended.
- Always fill your sample holders with samples when doing lyophilisation, part filled holders may not freeze.
- This method will not be able to lyophilise samples that are grossly insoluble in water – these will crash out once the organic solvent has been removed from the mixture.
- Never use Auto-Defrost and Drain on the final stages of a lyophilisation method – solvent vapour from the condenser may re-enter the samples and cause the powder to collapse. Feedback from some Genevac users suggests that this is worse when samples are TFA salts, where as HCl salts are reportedly more robust. These observations have not been proven. Only defrost the condenser once you have removed your samples from the system.

Drying Mixed High and Low Boiling Point Samples

The actual method that you will need depends on the solvents that you have in the mixture. We have outlined a mix of DMF or DMSO and DCM in this illustration. For other details of mixtures, please contact Genevac.

1. Use Variable Dri-Pure™ to prevent bumping
   Ramp from 750mbar to 70 mbar in 55 minutes
2. Remove the DCM, and collect it all in the cold trap
   Evaporate the DCM at 65mbar
3. Auto-Defrost and Drain – this will be a short defrost and will drain the organic solvent from the condenser, allowing high vacuum levels to be achieved for drying the high boiling point solvent.
4. Evaporate the high boiling point solvent
   Use full vacuum – end the method using heat flow
5. Dry any stubborn leftover solvent
   Use full vacuum for 1 hour
6. Auto-Defrost and Drain - this will be a full defrost and drain.

See appendix C for programming details.
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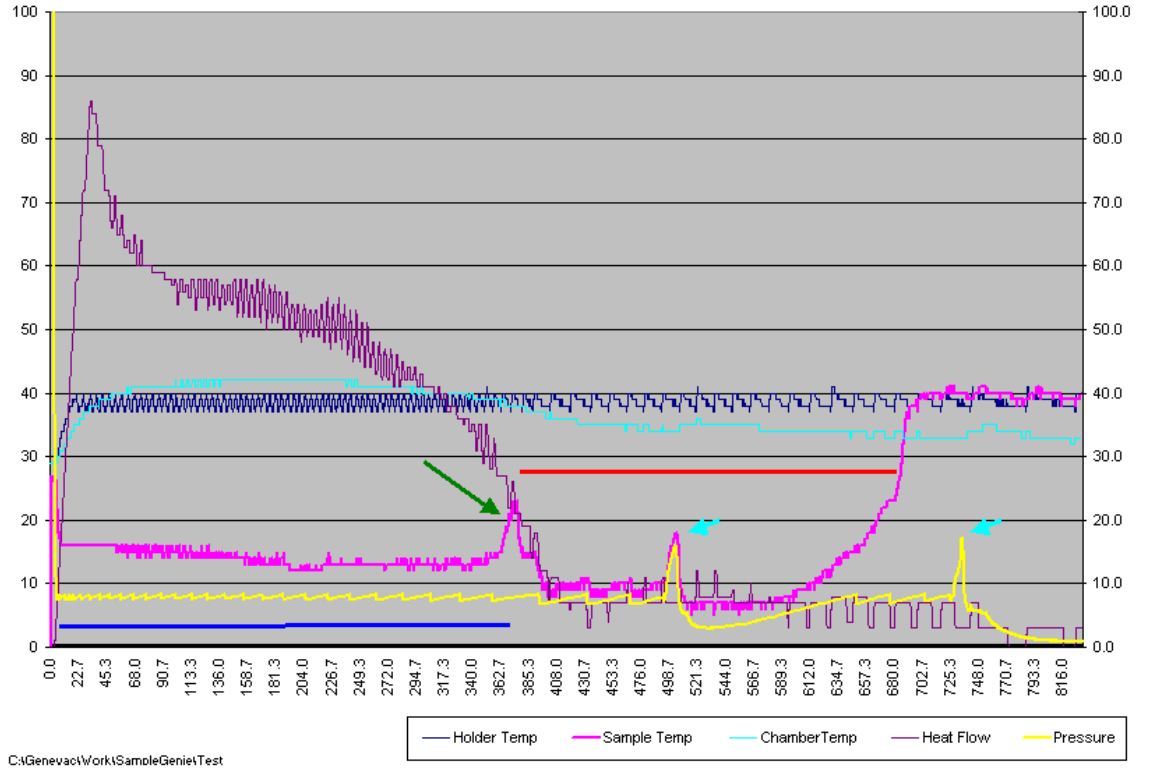
Use of SampleGenie™ for Lyophilisation

When the SampleGenie™ is used to lyophilise samples, the most important fact is how to determine whether the solvent is still in the flask or in the vial. So the time required to concentrate the water becomes a crucial point. This is an easy task if all the samples are the same solvent and volume but when it varies it will becomes a little tricky!

Polypropylene adaptors are especially designed to overcome this problem because they have a poor heat transfer ability, therefore the rate of evaporation in the vial is greatly reduced. This is very useful when samples with HPLC fractions of varying solvent ratios are being lyophilised, the first sample to concentrate into the vial will not be dry by the time the slowest sample to concentrate enters the vial.

The graph below gives indicates the evaporation rate in the flask and vial in a polypropylene adaptor.



Graph 1

Starting with 60mls of water, running at 8mbar to dry. From the graph, you can see it takes about 7 hours to dry 55 ml of water in the flask (blue line) and around 5 hours to evaporate 5 ml of water in the vial (red line) with a polypropylene adaptor. The peak at 360 minutes is an indication of where the solvent level moves from the flask to the vial (green arrow). For those of you who wonder what the two peaks are in the final drying stage highlighted by the two light blue arrows – the pressure rises because when an HT-4X has been running at 8mbar for a long time, the system does a short defrost every 4 hours to ensure that the vacuum tube to the condenser does not freeze.

Results are promising, with high recoveries, starting with 15mg sample in solution we achieved recoveries of 14mg and above, therefore are over 93%.

The different stages for a lyophilisation method

1. Evaporation of acetonitrile followed by a short defrost and drain
2. Concentration of water
3. Lyophilisation - Unlike the fast lyo procedure, we don't apply heat.

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How to Get Good Results

What you need to know:

- The maximum volume of acetonitrile and water
- The volume of the vial
- Total volume

Example 1- Lets look at a situation where the samples are in 50/50 acetonitrile:water and a volume of 60 ml, 8 flasks, volume of vial – 5ml up to the top. Refer to Graph 2 below to see what's happening

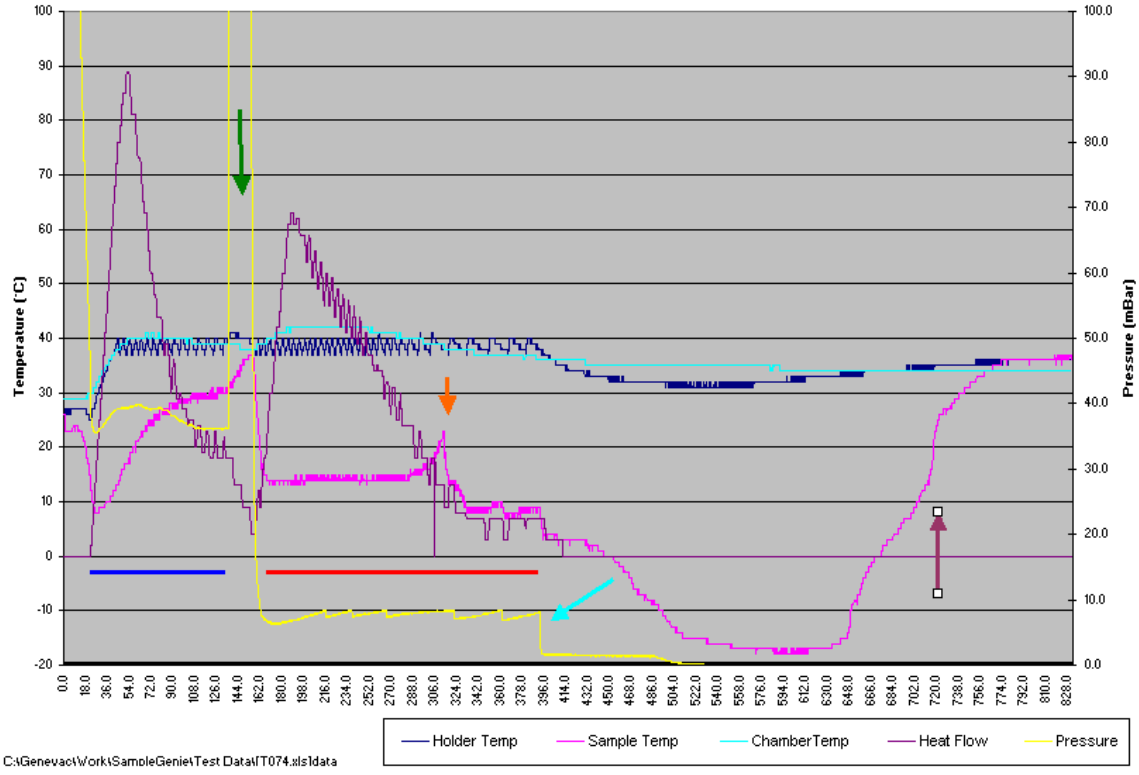
1. *Evaporation of acetonitrile* – No tricks! To evaporate 30 ml of acetonitrile it will take about 2 hours at 40°C (blue line) then auto defrost and drain (green arrow).

2. *Concentration of water* – 30 ml is the total volume but the vial will contain 5 ml so the total volume in the flask we need to *evaporate* is 25 ml.
   This can be achieved either by using heatflow or by determining the time as explained below.

   We know that it will take 7 hours to evaporate 55 ml of water so it will take 190 minutes (3.18 h) to evaporate the 25 ml. Let s take it as 4 hours (red line). In this case it seems that we have over estimated a little as the peak (orange arrow) shows when the liquid level dropped into the vial. This is no bad thing though since we want he level in the vial to be below the neck of the vial. 4hrs will ensure this.

   From the work done at Genevac heatflow seems like a better option but if the solvent level is too high when it terminates, link another short run (to concentrate the water) before pulling a full vacuum.

3. *Lyo* - Full vacuum, no heat, over night. Starts at light blue arrow, complete at the purple arrow. Future runs can have the correct lyo time set, add 2 hours to the time shown by the arrow to be safe!



Graph 2

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Example 2-
Say your customer uses volumes less than 60 ml and typically between 30:70 acetonitrile:water to 70:30. The vial, we assume, will be 5 ml.

1 Evaporation of acetonitrile – A sample containing 70:30 acetonitrile:water will have 42 ml of acetonitrile. To evaporate this will take about 2.30 h, and followed by defrost and drain. Note here we chose the highest Acetonitrile level

2 Concentration of water – Choose either heatflow or time.
If a sample is 70:30 water : acetonitrile there will be 42 ml of water So 42 -5 = 37 ml and this will take about 282 minutes (lets say 5 hours). It is unlikely that the sample with only 18ml water will completely dry because evaporation rates are so slow in the vial. Note here we chose the highest Water level

3 Lyo - Full vacuum, no heat, over night. Work out the correct time as per example 1

Notes on Lyophilisation:

Using Methanol
If using methanol in place of acetonitrile then several minor adjustments will have to be made to the method to accommodate this.

1. Set the pressure to evaporate methanol to 45mbar, rather than 40mbar
2. The evaporation time for 1ml of methanol is 50% more than for 1ml of acetonitrile

Samples stick to the SampleGenie Flask
This problem is encountered when the organic solvent evaporates and the sample is grossly insoluble in water, therefore it crashes out and sticks to any surface it can find. Surface treatment of the glass with a silanising agent is helpful, however, it is better to prevent crashing out in the first place. A method has been researched and published by Genevac which shows how to add a volume of 1,4-dioxane to the sample before evaporation commences that will keep it suspended until all the sample is in the vial. This work was originally done in the Rocket Evaporator, but the method will transfer to the HT series.

If you have problems, please contact applications@genevac.com

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Appendix A

Programming Guide for Drying HPLC Fractions

	Drying HPLC Fractions		
Run number	1	2	3
Run Data Locked	N	N	N
Next Run Number	2	3	0
Run Name	Fractions	Fractions part 2	Fractions part 3
Sample Holder Type	Your Holder	Your Holder	Your Holder
Sample Volume ml	Your Volume	Your Volume	Your Volume
Solvent	MeCN or MeOH & H_20	MeCN or MeOH & H_20	MeCN or MeOH & H_20
SampleGuard Control Temp C	40	40	40
SampleGuard Control Channel	1	1	1
CoolHeat Enable Pressure	50	50	50
Chamber Temperature control	preheat to 25	preheat to 30	preheat to 30
Rotor Speed	High	High	Low
Heat-Off Option	Elapsed Time	Heat Flow	Elapsed Time
Heat-Off Elapsed Time	*ask for time*	-	01:00 - 03:00
End-of-Run Option	Elapsed Time	-	Elapsed Time
End-of-Run Elapsed Time	*ask for time*	-	01:00 - 03:00
Auto Defrost & Drain	Y	N	Y
Pressure Control Regime	Variable Dri-Pure	Controlled Pressure	Full Vacuum
Dri-Pure Start Pressure (mbar)	175	-	-
Dri-Pure End Pressure (mbar)	40 for MeCN 45 for MeOH	-	-
Dri-Pure Ramp Time	20	-	-
Final Control Pressure	40 for MeCN 45 for MeOH	8	-
Notes	Remove organic phase	Remove most of the aqueous phase	Remove final “stubborn” solvents

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Appendix B

Programming guide for fast lyophilisation of HPLC fractions

	Lyophilising HPLC Fractions			
Run number	1	2	3	4
Run Data Locked	N	N	N	N
Next Run Number	2	3	4	0
Run Name	Fractions Lyo	Fractions Lyo part 2	Fractions Lyo part 3	Fractions Lyo part 4
Sample Holder Type	Your Holder	Your Holder	Your Holder	Your Holder
Sample Volume ml	Your Volume	Your Volume	Your Volume	Your Volume
Solvent	MeCN or MeOH & H20	MeCN or MeOH & H20	MeCN or MeOH & H20	MeCN or MeOH & H20
SampleGuard Control Temp C	40	0	0	40
SampleGuard Control Channel	1	1	1	1
CoolHeat Enable Pressure	50	50	50	50
Chamber Temperature control	preheat to 25	preheat to 0	preheat to 0	preheat to 30
Rotor Speed	High	High	Low	Low
Heat-Off Option	Elapsed Time	Elapsed Time	Elapsed Time	Elapsed Time
Heat-Off Elapsed Time	*ask for time*	0:00	00:00	05:00
End-of-Run Option	Elapsed Time	Elapsed Time	Elapsed Time	Elapsed Time
End-of-Run Elapsed Time	*ask for time*	0:30	01:00	10:00
Auto Defrost & Drain	Y	N	N	N
Pressure Control Regime	Variable Dri-Pure	Controlled Pressure	Full Vacuum	Full Vacuum
Dri-Pure Start Pressure (mbar)	175	-	-	-
Dri-Pure End Pressure (mbar)	40 for MeCN 45 for MeOH	-	-	-
Dri-Pure Ramp Time	20	-	-	-
Final Control Pressure	40 for MeCN 45 for MeOH	8	-	-
Notes	Remove MeCN	Cool the water	Freeze the water	Lyophilise the water

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Appendix C

Programming guide for mixtures of high and low boiling point solvents

	Drying High - Low Mixtures		
Run number	1	2	3
Run Data Locked	N	N	N
Next Run Number	2	3	0
Run Name	High - Low Mix	High - Low Mix part 2	High - Low Mix part 3
Sample Holder Type	Your Holder	Your Holder	Your Holder
Sample Volume ml	Your Volume	Your Volume	Your Volume
Solvent	DMSO or DMF & DCM	DMSO or DMF & DCM	DMSO or DMF & DCM
SampleGuard Control Temp C	40	40	40
SampleGuard Control Channel	1	1	1
CoolHeat Enable Pressure	50	50	50
Chamber Temperature control	preheat to 15	preheat to 40	preheat to 30
Rotor Speed	High	High	Low
Heat-Off Option	Elapsed Time	Heat Flow	Elapsed Time
Heat-Off Elapsed Time	ask for time	-	00:00
End-of-Run Option	Elapsed Time	-	Elapsed Time
End-of-Run Elapsed Time	ask for time	-	01:00
Auto Defrost & Drain	Y	N	Y
Pressure Control Regime	Variable Dri-Pure	Full Vacuum	Full Vacuum
Dri-Pure Start Pressure (mbar)	750	-	-
Dri-Pure End Pressure (mbar)	70	-	-
Dri-Pure Ramp Time	55	-	-
Final Control Pressure	65	-	-
Notes	Remove MeCN	Dry high BP solvent	Final Drying

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